ON THE PHARMACOLOGICAL ACTION OF SOME PHTHALEINS AND THEIR DERIVATIVES, WITH ESPECIAL REFERENCE TO THEIR BEHAVIOR AS PURGATIVES. I.

SLEEPING SICKNESS BUREAU
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(From the Pharmacological Laboratory of the Johns Hopkins University)

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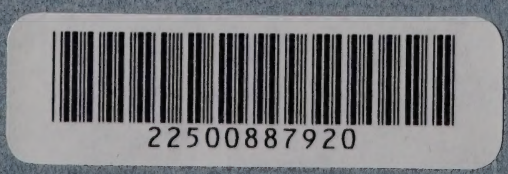

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ON THE PHARMACOLOGICAL ACTION OF SOME PHTHALEINS AND THEIR DERIVATIVES, WITH ESPECIAL REFERENCE TO THEIR BEHAVIOR AS PURGATIVES. I.

JOHN J. ABEL and L. G. ROWNTREE

From the Pharmacological Laboratory of the Johns Hopkins University

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This and a later paper will deal with the comparative pharmacological action of a number of phthaleins and their derivatives. It has been our purpose to study the influence of substitution in various parts of the molecule upon the pharmacological and physiological properties of the phthaleins, more especially upon their excretion, reabsorption and purgative action.

Aside from these problems of theoretical and practical interest, we have had in view the possible attainment of a therapeutic end, namely, to find a serviceable hypodermic purgative. We must defer for a later paper a consideration of the question of how far and in what respect changes in chemical constitution affect pharmacological action in this series of drugs.

Vamossy[1] has shown that phenolphthalein is a mild purgative, but little absorbable from the digestive tract, and quite devoid of toxic action. This phthalein has been used by us as a standard in our pharmacological comparisons.

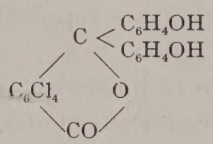
The compounds that we have compared with phenolphthalein are phenoltetrachlorphthalein, tetrabromphenoltetrachlorphthalein, phenoltetrachlorphthalein diacetate, tetrabromphenoltetrachlorphthalein diacetate, phenolphthalin, ethylester of phenolphthalin, ethylester of tetrabromphenolphthalin, tetrabromphenolphthalein, hydroxycarboxyethylester of phenolphthalein, fluorescein, disodium salt of tetraiodophenolphthalein, phenolsulphonphthalein, and sulphonfluorescein.

We hope to extend this list and to include in our further studies one or more examples of the phthalidines, phthalideines and phthalides and perhaps other compounds not far removed from these in constitution.

[1] Therapie der Gegenwart. Jahrg., xliii, 203, 1902.

The first four compounds in the above list we owe to the kindness of Professor Orndorff[2] of Cornell University. In April, 1908, we received a letter from him stating that he had prepared a number of phenolphthalein derivatives and inquiring if we would be interested in them from a pharmacological point of view. We wish to express our thanks for the generosity with which he has responded to our repeated demands for supplies of material. The phenolsulphonphthalein used by us was kindly presented by President Remsen, while the tetrabromphenolphthalein, its hydroxycarboxyethylester, the phenolphthalin and its derivatives were presented by Dr. E. A. Slagle. Prof. S. F. Acree has also been of service in supplying material for our researches. To these gentlemen we would offer our thanks for their generous assistance.

Phenoltetrachlorphthalein,



In studying the compounds prepared by Professor Orndorff our chief interest has centered in this substance. Its physical and chemical properties present such similarities to those of phenolphthalein that one is surprised to learn that there are certain well-marked pharmacological differences between them. Like phenolphthalein it is an odorless, tasteless, crystalline compound, insoluble in water, and forming deeply colored, hydrolyzable salts with alkalies. Its ionization constant has not yet been determined, but its avidity as an acid cannot be far removed from that of phenolphthalein, inasmuch as solutions of its salts (Na or K) are promptly decolorized on the addition of serum or by contact with animal tissues. In this respect its salts differ in no way from those of phenolphthalein.

The two compounds have,[3] on the whole, a very similar pharmacological action.

[2] Orndorff and Black, Amer. Chem. Jour., xli, 349, 1909.

[3] The phenolphthalein used by us was obtained from C. A. F. Kahlbaum. In regard to the phenoltetrachlorphthalein and other compounds of Orndorff, it was stated that all samples sent to us had been carefully analyzed and were of the highest attainable purity.

I. BOTH ARE NON-IRRITANT WHEN APPLIED LOCALLY

The drugs were dusted into the conjunctival sac of the rabbit without producing congestion or signs of irritation during the period of observation of twelve hours. Suspensions in very dilute alcohol injected beneath the skin failed to cause any infiltration. Superficial wounds in animals when treated with the drugs as dusting powders healed readily and quickly.

II. THE FREE SUBSTANCES IN BOTH CASES ARE NON-IRRITANT, YET SOLUTIONS OF THEIR ALKALI SALTS APPLIED SUBCUTANEOUSLY ACT AS IRRITANTS UNLESS THEY ARE HIGHLY DILUTED

This results from the fact that such salts, formed as they are by neutralizing a very weak acid with a powerful base, are strongly hydrolyzed in aqueous solution and hence act upon the tissues like solutions of the caustic alkalies. When such solutions[4] are injected in large amounts under the skin of dogs in regions where the connective tissue is loose, a sterile infiltration with necrosis of fatty tissue results, therefore after a few trials this method of administration was discarded. It appeared to us that solutions of the disodium salt of tetrachlorphthalein were more irritating to the subcutaneous tissues of dogs than were those of the corresponding salt of phenolphthalein.

Fleig[5] has prepared a salt of phenolphthalein which he has named sodophtalyl and which is being used with success, according to his account, as a hypodermic purgative in some of the clinics of Montpellier. Fleig's description of the chemical properties and methods of preparation of his sodophtalyl are so meagre that no definite statement can be made in regard to its constitution. We surmise, however, that it is identical with the disodium salt of phenolphthalein as prepared by Meyer and Marx.[6] We await with interest further clinical accounts of the action of Fleig's salt when administered subcutaneously, for we fail to see how pain and local irritation can be absent after its use in this way.

[4] These solutions were made as nearly isotonic with blood as possible by the help of sodium chloride.

[5] Archives Internat. de Pharmacodyn. et de Thérapie, xviii, 327.

[6] Ber. d. deutsch. chem. Gesellsch., xli, 2446, 1908.

III. BOTH SUBSTANCES HAVE A LOW TOXICITY

Vamossy,[7] Unterberg,[8] Wenhardt,[9] Kastle,[10] Fleig,[11] Elmer,[12] and Gillette[13] have shown that this statement is true for phenolphthalein, while our own experiments prove that it holds also for its tetrachlor derivative. Neither drug when administered by mouth is absorbed in any but small amounts from the digestive tract, so that their toxic action must be determined by subcutaneous or intravenous administration. Fleig has shown that considerable amounts of phenolphthalein in the form of its sodium salt may be injected directly into the blood stream of an animal without noticeable injury. We have found that phenoltetrachlorphthalein is equally innocuous when administered in this way.

The aqueous solutions of the disodium salt of both phenolphthalein and its tetrachlor derivative, whether intended for subcutaneous or intravenous injections, were always prepared as follows: An excess[14] of the phthalein was suspended in 90 cc. of a weak solution of sodium chloride, say $\frac{N}{2\,3}$, 10 cc. of 2N NaOH solution were added and the mixture was boiled in a flask connected with a reflux condenser for twenty minutes, then freed from the undissolved phthalein by filtration. In the case of phenolphthalein this gives a 3.62 per cent solution of the sodium salt and in the case of its tetrachlor derivative the solution contains 5 (more accurately 4.99) per cent of the sodium salt. Neglecting hydrolytic dissociation, the degree of which could be estimated only roughly, solutions thus prepared do not differ much from blood serum in tonicity. Carbonic acid must not be allowed to come into contact with these solutions. Even when solutions are protected against the entrance of this gas, precipitation of the free phthaleins soon occurs in consequence of the loss of alkali from union with the silicates of the containing flasks.

[7] Therapie der Gegenwart. Jahrg., lxiii, 1902.

[8] Therapie der Gegenwart. Jahrg., lxiii, 203, 1902.

[9] Die Heilkunde, vi, 212,

[10] Bull. No. 26, p. 23, Hygienic Laboratory, U. S. Public Health and Marine Hospital Service.

[11] Loc. cit.

[12] Medical Record, November 14, 1908.

[13] Jour. Amer. Med. Assoc., li, No. 21, p. 1783.

[14] Of the tetrachlor derivative 4.8 grams, of phenolphthalein 3.4 grams were taken.

Experiment I. 11/19/'08. Dog weighing 12.15 kg. received by the saphenous vein (local anæsthesia with cocaine) 8 cc. of a 5 per cent solution of the disodium salt of phenoltetrachlorphthalein (0.4 gram). Within ten minutes it had fully regained its former playfulness and exhibited absolutely no untoward symptoms.

The observations extended over several days but revealed nothing more than the data embodied in the following table:[15]

DATE.	CONDITION OF DOG.	PURGATION.	DRUG IN STOOL.	DRUG FREE IN URINE.	DRUG CONJUGATE IN URINE.	REMARKS.
11/20....	normal	— no fæces	—	+	+	The conjugated form* was present in large amount on the 20th
21....	"	— no fæces	—	—	—	
22....	"	+	+	—	—	
23....	"	+	+	—	—	
24....	"	+	+	—	—	
25....	"	—	—			

*The drug is excreted in two forms: in the one form it gives the well known red color on the addition of a free alkali, while in the second it must be hydrolyzed by boiling with an acid before it will give the color reaction with alkali.

Experiment II. 11/27/'08. A dog weighing 14.6 kg. which had been under observation, on a constant diet, for two weeks, received into the saphenous vein, under local anæsthesia, 25 cc. of a 2½ per cent solution of the disodium salt of phenoltetrachlorphthalein (0.625 gram). As before, no symptoms of any kind were noticed. The results in respect to purgative action and excretion of the drug in the fæces and urine are here tabulated:

DATE.	CONDITION OF ANIMAL.	PURGATION.	DRUG IN FÆCES.	DRUG IN URINE.	
				In free state.	In conjugated form.
11/28....	normal	+	+	—	+
29....	"	+	+	—	—
30....	"	+	+	—	—
12/ 1....	"	—		—	—
2....	"	no fæces		—	—

[15] In this and in other tables of this paper, the sign + indicates a positive result, the sign — indicates a negative result.

Much larger quantities were given, likewise intravenously and under local anæsthesia in experiments that were made with another purpose than that of studying the toxicity of the compound, yet here also no untoward effects were observed. Thus, in one experiment, a dog weighing 6 kg. was given 35 cc. of a 2.51 per cent solution of the sodium salt of the tetrachlor derivative in this way without showing any signs of discomfort during the four hours following the injection, at which time the animal was killed.

Many animals have received once a week, for a number of weeks in succession, without showing any signs of ill health, subcutaneous injections of solutions of this and certain other phthaleins made by dissolving them in olive oil. In numerous autopsies we have never observed any lesions that could be referred to these compounds.

Inasmuch as the liver is the organ on which falls almost the entire burden of excreting these substances, we have thought it worth while to make a histological examination of this organ in the case of a dog that had been the subject of numerous experiments throughout a period of three months. The animal was in perfect condition when it was killed. In the course of three months it had received phthalein by the mouth and in the form of injections as follows:

1. *Subcutaneous injections:*
   - *a.* 0.4 gram phenoltetrachlorphthalein, 0.002 gm. eserine sulphate in 20 cc. neutral olive oil.
   - *b.* 0.24 gram dibromphenolphthalein in sodium carbonate solution.
   - *c.* Phenolphthalein 0.415 gram in 20 cc. olive oil.
   - *d.* Acetyl derivative of tetrabromphenoltetrachlorphthalein, 500 gr. in 10 cc. olive oil.
2. *Intravenous injections:*
   5 cc. of a 3.6 per cent of a solution of the disodium salt of phenolphthalein in isotonic saline solution.
3. *Administration by mouth:*
   - *a.* 1 gram of the disodium salt tetraiodophenolphthalein.
   - *b.* 1 gram of phenolphthalein.
   - *c.* 1 gram of dihydroxy ester of tetrabromphenolphthalein.

Pieces of the liver of this dog were placed in Zenker's fluid and sent to the Pathological Laboratory of this University for examination. Sections were made which Dr. Whipple kindly examined for us. The report "normal" was returned, a histological confirmation to our clinical observation that was most welcome.

In corroboration of the results just described, we would state that Dr. Whipple also examined for us all of the abdominal and thoracic viscera of a second dog that had received four injections, at intervals of a week, of the tetrachlor derivative dissolved in oil, the total quantity injected being 1.3 gm. All of the organs were reported as normal on macroscopical examination. Under the microscope, sections of the liver showed a slight excess of fat, in correspondence with the high state of nutrition of the animal, which had increased in weight during the time of the experiment.

Our observations, therefore, lead us to conclude that phenoltetrachlorphthalein like phenolphthalein itself is a substance of very low toxicity. It may be administered either by the mouth or, for a time at least, in the form of subcutaneous injections without fear of untoward results.

IV. EFFECT ON BLOOD-PRESSURE AND ACTION ON RED BLOOD CELLS

Inasmuch as we were obliged to make intravenous injections in order to compare the toxicity of the two compounds (phenoltetrachlorphthalein and phenolphthalein), we have thought it advisable to study the effect of these substances on blood-pressure and on coagulation time and also to determine whether they cause any degree of hæmolysis.

Good proof of the absence of any marked hæmolytic power was obtained by collecting the blood of dogs from the carotid artery within half an hour after the intravenous injection of large quantities of the sodium salt, and allowing the blood to stand upon ice until the serum separated from the clot. In experiments of this kind a clear, straw-colored serum was obtained containing no dissolved hæmoglobin.

Similarly, negative results were obtained in test-tube experiments in which red corpuscles of dog's blood were suspended in serum of dog's blood to which solutions of the sodium salts (approximately isotonic) of both the tetrachlor derivative and phenolphthalein were added. As the salts of these compounds undergo hydrolytic dissociation, it is evident that the effect of free alkalies will be obtained in case too large a quantity of the salts is added to a suspension of corpuscles. The use of serum as a medium of suspension no doubt

protected the corpuscles against this injurious action, but it also served to bring our tests into line with the actual clinical circumstances in which the drug was used, namely, by intravenous or subcutaneous injection.

In our experiments we proceeded as follows: Two cubic centimeters of the solution of the sodium salts of the two phthaleins were added separately to 13 cc. of serum from dog's blood. When the mixture is shaken the red color of the phthalein salt is at once discharged and without precipitation of the free phthaleins. Of this solution in serum 5 cc. were added to 2 cc. of a 2 per cent suspension of dog's red corpuscles. Even when tubes thus prepared were kept in the thermostat at 37.5° C. for eighteen hours, not the slightest degree of hæmolysis was produced. The action of larger quantities of these phthaleins on red corpuscles was not tested, as it seemed certain that under no circumstances would the blood ever be exposed to even this concentration. It was also determined that neither of these phthaleins altered the coagulation time of the blood. The tests relating to this point were made with Boggs' coagulometer.

Effect on blood-pressure. The carotid artery of a dog weighing 6 kg. was connected with a mercury manometer in the usual way, and varying quantities of solutions of the disodium salt of phenolphthalein and its tetrachlor derivative were injected into the saphenous vein. These solutions were approximately isotonic with the blood, having been made up with the help of a dilute saline solution. The solution of the chlor derivative contained 5.2 per cent of the disodium salt, that of phenolphthalein contained 3.62 per cent of the corresponding disodium salt. The result may be briefly summarized as follows: If 5 or 10 cc. of the solution of either salt be injected *very rapidly* into the blood stream, a sharp fall in blood-pressure will result, varying in degree from 20 to 50 mm. of mercury. The chlor body is probably the more effective of the two in causing this fall of pressure. The fall of pressure is of a fleeting character, lasting not more than ten seconds, and is immediately followed by a return to the normal which is regained in less than a minute. After repeated rapid injections the fall after each injection is still well marked, but on the return, the rise continues to a point above the normal and the pressure is maintained for some time at a point which lies 20 mm. higher than at the

start. Equally rapid injections of a dilute solution of sodium hydroxide in 0.75 per cent sodium chloride solution also induce a fall of pressure and quick return to the normal, but in this case the low point is never more than 25 mm. below the normal. Slow injections, that is, those in which two minutes are consumed in injecting 10 cc. of the solution into a vein, are not followed by a fall in the arterial pressure. These slow injections when repeated two or three times have the same ultimate effect on the pressure as the rapid injections, that is to say, they induce a rise of the arterial pressure of from 15 to 25 mm. Hg which persists for fifteen minutes after the last injection. In conclusion, we may say that the arterial pressure is only lowered by these drugs when they are injected into the blood current with undue rapidity, and even in that case the fall is of the most transient character and, further, a slight rise of arterial pressure is caused by the presence of large quantities of the drug in the vascular system.

It is fair to assume that when the drugs are injected subcutaneously as in experiments presently to be described, no depressant action whatever on the blood-pressure will be produced.

It may be stated in this connection that Tunnicliffe[16] has made observations upon the effect of the administration of phenolphthalein upon the arterial blood pressure with the sphygmomanometer of Mosso. He believes that a reduction of arterial pressure was induced by phenolphthalein which lasted practically until the purgative effect of the dose given ceased, but this reduction could not have been of any considerable magnitude, since he comments as follows upon the result of his experiment: "This result is of importance in that it points to the conclusion that purgen (phenolphthalein) will not be of service in cases in which we wish to obtain the secondary depressant effect of a purgative upon the circulation; yet, nevertheless, it will probably be useful precisely in those cases in which we wish to avoid this depressant action. Especialy is it advisable to do this in cases of *morbus cordis* with dilated and degenerate heart. In such cases, especially if associated with albumin in the urine, a non-depressant and non-irritating purgative is a desideratum."

[16] Brit. Med. Journ., October 18, p. 1224, 1902.

V. EXPERIMENTS TO DETERMINE WHETHER PHENOLTETRACHLORPHTHALEIN HAS BACTERICIDAL OR ANTISEPTIC PROPERTIES

Owing to the rapidity with which non-infected wounds heal when they are treated with this substance, an effort was made to ascertain if it is possessed of bactericidal or antiseptic properties. Gelatin and agar were prepared, but before sterilization, a few drops of oil containing phenoltetrachlorphthalein was added to each tube and intimately mixed by shaking. The medium was slightly alkaline and became red on this account. The tubes were sterilized and cooled, but some of the oil was found floating on the surface of the slant after solidification. This was poured off and the tubes were re-sterilized, and finally a fair culture medium was obtained. The tubes were inoculated with B. proteus and a luxuriant growth was obtained.

Bile, obtained from the gall-bladder of a dog which had been injected subcutaneously with the tetrachlorphthalein, contained this body in abundance. A tube containing such bile was incubated for twenty-four hours and from it agar tubes were inoculated and plates poured. Numerous colonies developed in our agar plates within twenty-four hours.

These experiments are sufficient to demonstrate that this substance possesses little or no antiseptic or bactericidal value.

VI. THE INFLUENCE OF THE TETRACHLOR DERIVATIVE ON THE RATE OF FLOW OF BILE AND PANCREATIC JUICE

Fleig[17] has endeavored to show that phenolphthalein causes a slight increase in the flow of bile, of pancreatic juice, and of the succus entericus. His experiments were made on dogs with temporary fistulæ, and extended over a short period of time only, two hours being allowed for the experiments on the rate of excretion of the biliary and pancreatic fluids. Tunnicliffe[18] infers from his clinical results with phenolphthalein that it has apparently no influence on the secretion of bile. He administered the drug in purgative doses to two children with simple catarrhal jaundice. The motions were clay-colored as before, though purgation was produced. Numerous

[17] Loc. cit., p. 359.

[18] Brit. Med. Journ., ii, 1224, 1902.

investigators[19] have shown that the variations that normally occur in the flow of bile are so considerable that no deductions as to the influence of a drug on the rate of secretion can be drawn from a few experiments of short duration on animals. Although fully aware of this fact, we have, nevertheless made one experiment on an anæsthetized dog with temporary fistulæ (biliary and pancreatic) in order to learn whether our drug has any immediate effect on the rate of flow of bile or of pancreatic juice. It was our intention to investigate the question more carefully, using dogs with permanent fistulæ in case the results obtained made it appear probable that the tetrachlor derivative exerted a marked action on the rate of flow of these fluids. In an experiment which lasted for nearly three hours 35 cc. of a 2.5 per cent solution of the sodium salt were injected intravenously in three portions. This considerable quantity of the tetrachlor derivative had no marked influence on the rate of flow of the secretions studied. The variations in flow were inconstant; thus, after the first injection the flow of bile rose from 0.2 cc. in ten minutes to 0.4 cc., the increased rate lasting for thirty minutes. Following the injection of a second dose of the drug the biliary flow returned at once to its former rate and finally fell below the normal. It will be seen, therefore, that we have no proof that our substance acts as a cholagogue. This point can only be determined with certainty by experiments of long duration on dogs with a permanent fistula.

In the above experiment the interesting fact was brought to light *that the tetrachlor derivative is excreted in the conjugated form in the bile before it appears in the "free" state.* Within ten minutes after the first injection the drug was excreted in the first-named combination, while twenty minutes elapsed before it could be detected by the addition of alkalies ("free" form).

VII. PURGATIVE ACTION OF PHENOLTETRACHLORPHTHALEIN

Before entering into a description of experiments under this head, it is necessary to outline briefly our method of treating dogs, and to define what constitutes purgation as the term is here used. In our

[19] See E. Stadelmann, Therapeut. Monatshefte, 1892, pp. 512 and 562; F. Pfaff and A. W. Balch, Journ. Exp. Med., ii, 49, for a discussion of this point.

first experiments in this field, which we made in April, 1908, we used rabbits, but preliminary experiments with a number of purgatives, both saline and vegetable, led us to discard this animal as unsatisfactory and untrustworthy in responding to this class of drugs. The dog on the other hand, while responding less readily to the phthaleins than man, is nevertheless well adapted to a research such as ours. Healthy bitches of medium size were selected, placed in separate cages and fed with known amounts of meat, with an occasional allowance of corn bread, and received water *ad libitum*. No experiments with dogs were undertaken until the fæces became well formed, clay-colored, dry and friable. Daily examinations were made into these particulars at a stated hour and each animal was led about in the open each day for a stated time. During the time when the dogs were under treatment with the drug the rules in respect to food and exercise were kept in force, and the fæces were collected daily, weighed, examined in regard to color and consistence, and tested for the presence of the drug. *When the fæces had changed from the clay-colored, dry and friable condition of the fore period to a brown or black, moist, homogeneous, sticky or mushy consistence we considered that our drug had acted as a laxative.* One of the best criteria for the estimation of the first onset of purgation in the dog is the disappearance of the granular and friable state of the fæces and the appearance of homogeneous, agglomerated, moist and sticky stools. With an increase in the purgative action a more mushy condition of the fæces results, and this may give place to the watery stools of diarrhœa, a condition which was only very infrequently observed in our experiments. In all instances of examination of the urine only samples obtained with the catheter were used.

When phenoltetrachlorphthalein is given to dogs by mouth in doses of 1 gram, it induces, in every instance, a mild purgative action, which continues for two, and sometimes for three days. *The fæces are not voided more frequently but become darker colored and softer in consistency, and contain the drug in large amounts.* Phenolphthalein given in equal doses acts in the same manner, at least in so far as its behavior as a purgative is concerned.[20] With this method of adminis-

[20] Vamossy has stated that phenolphthalein will not purge dogs even when given in doses of five and six grams. This investigator evidently expected to obtain

tration the chlor derivative is never excreted in the urine, either as such or in the form of a conjugated derivative, while phenolphthalein under the same conditions is generally to be detected in this secretion.

One of us (R.) took 0.4 gm. of the tetrachlor derivative and found that this quantity caused the appearance of large, unformed, soft and pulpy stools at the expiration of twelve hours. This result was obtained without pain or discomfort of any kind and was not followed by constipation.

Similar results were obtained with this drug in trials made in medical practice during the past summer, but the data thus obtained will be given later in a separate paper.

The experiments thus far made show, therefore, that phenoltetrachlorphthalein is no less efficient as a purgative when administered by mouth than phenolphthalein itself, and that it has the added advantage of not being excreted by the kidneys.

VIII. ON THE EFFICACY OF PHENOLTETRACHLORPHTHALEIN AS A HYPODERMIC PURGATIVE

It will readily be granted that practical medicine stands in need of an efficient hypodermic purgative, one which will produce the desired results without inducing local irritation at the point of injection or undesirable concomitant actions elsewhere in the organism. The worker in the laboratory has at his disposal a number of glucosides, alkaloids and salines which will act promptly when administered to animals in this way, but their untoward effects are such as to make their use in practical medicine impossible. Nevertheless, two alkaloids are now being used to a limited extent in medical practice, namely, eserine[21] (physostigmine) and apocodeine[22]. The first named

watery stools from dogs after administering this laxative. *We have assumed that a laxative action is exerted when the stools change from the hard friable condition which is normal for dogs to the moist, homogeneous and softer state described above.* This state is compatible with formed stools, though in the majority of the cases treated with the tetrachlor. derivative, the stools were mushy and poorly formed.

[21] Von Noorden: Berl. klin. Wochn., p. 1057, 1901. Packard: Phila. Med. Jour., May 24, 1902. Pankow: Zentralb. f. Gyn., June, 1904. Craig: Amer. Jour. Obstetrics. April, 1904. Amer. Jour. Obstetrics, September, 1904. New York Med. Jour., lxxxi, 527, 1905. Jewett: Brooklyn Med. Jour., xix, 42.

[22] Raviart et Bertin: Echo du Nord, December, 1902; cited from G. Giraud—Thése de Lyon, 1903. Heinze: Psychiatrisch Neurologische Wochenschrift, v, 297. Dixon: Brit. Med. Journ., ii, 1244, 1902.

of these is a powerful alkaloid which must be used judiciously and is of service in overcoming tympanitis, in warding off threatened intestinal paresis, or in dealing with the obstinate cases of intestinal atony that sometimes follow parturition; the second is of wider applicability with fewer untoward effects, but according to the statistics of Heinze, it cannot be relied upon in more than 47 per cent of the cases. In hospitals for the insane, for epileptics and feeble minded, occasions frequently arise when a hypodermic purgative is needed. The case books of such institutions contain numerous accounts of patients who refuse to take drugs by mouth and who offer resistance to the administration of enemata for the relief of obstinate constipation. In conditions of the digestive tract when administration by mouth is impracticable, in comatose states, in cases where tolerance has been established to purgatives given by mouth, and especially in the field of abdominal surgery a hypodermic purgative with a prolonged action and devoid of any marked influence on other organs than the lower bowel is greatly needed.

We have already alluded to the fact that M. C. Fleig[23] has introduced into medical practice as a hypodermic purgative a soluble derivative of phenolphthalein which he has called "sodophtalyl." He says that this salt has been used hypodermically in doses of 0.2 to 0.4 gm. with success in the medical and gynecological clinics of Montpellier. If 3 per cent[24] solutions, made up with sodium chloride to be isotonic with the blood, are used for these injections, as much as $13\frac{1}{3}$ cc. of fluid must be injected in order to attain the upper dose of 0.4 cc. In case the solutions are weaker than 3 per cent, correspondingly larger quantities of fluid must be injected. This in itself is no objection to the clinical use of this drug. In case there is no irritation in consequence of hydrolysis of the "sodophtalyl," a point to which we have already alluded, this compound may indeed meet the expectations of its inventor.

Several months before the appearance of Fleig's article we made experiments on the behavior of phenolphthalein and of its tetrachlor derivative when used as a hypodermic purgative. For this purpose a search was made for a neutral and non-irritating solvent which

[23] Fleig, loc. cit. p. 361.
[24] Fleig, ibid., p. 341.

would take up these substances in sufficient amount for use in subcutaneous administration. We have as yet found no better non-irritating solvent than olive oil which has been freed from every trace of oleic acid.

After the appearance of Fleig's paper, we began to inject solutions of the sodium salts of phenolphthalein and its chlor derivative, the preparation of which has already been described. We soon found that these solutions act as irritants when injected in considerable amounts into the subcutaneous tissue. Small quantities may be injected without causing sterile infiltration if massage be applied to the area of injection. One of us (R.) injected 2 cc. of a 2.5 per cent solution of the sodium salt of the chlor derivative under the skin of his right thigh, and found that the operation was followed by pain and slight signs of local infiltration which persisted for several days. Every precaution had been taken against infection and we can only conclude that the above results were due to the irritating action of the hydroxide liberated in the hydrolytic dissociation of the sodium salt of these phthaleins.

The solutions in neutral olive oil were prepared as follows: The oil is slowly heated to 210 ° C., and when this temperature is attained finely powdered phenoltetrachlorphthalein is added with stirring, while the heating is continued for not longer than five minutes and the temperature not allowed to go over 220° C. In order to remove any trace of foreign matter or undissolved phthalein the oil solution is filtered hot into sterilized flasks which are closed with sterilized cotton and set aside for use. Olive oil thus treated will dissolve the chlor derivatives to the extent of 0.2 gm. to every 10 cc. of oil, while it will dissolve phenolphthalein to a slightly greater extent. In order to lessen the risk of saponification it has been our custom to prepare not more than 40 cc. of such a solution at a time, though we have no doubt that a larger volume of solution might be prepared at one operation. Analytical experiments in which the acidity of the oil was tested before and after exposure to heat, as in the preparation of our solutions, have shown us that no appreciable saponification of the oil takes place under these conditions.

With such solutions of the chlor derivative in oil a large number of experiments have been made; with solutions of phenolphthalein

the experiments have been fewer in number. The injections in the case of dogs were made into the loose tissue at the back of the neck a little forward of the shoulder-blade. In a series of fifty experiments in which the amount of oil solution injected varied from 20 to 30 cc., no instance of local irritation, injection, or infiltration occurred. In every instance also a laxative action could be recorded. *By this we mean that the stools lost their hard and friable condition and became moist and pasty in consistence.* Neither phenolphthalein itself nor any of the phthaleins mentioned in this paper gave equally good results when administered in this way. The following table gives the results of a single injection each of the tetrachlor derivative, of phenolphthalein and of tetrabromphenoltetrachlorphthalein into dogs, each animal receiving the same number of milligrams (23) pro kg. of body-weight.

It will be observed that the tetrachlor derivative caused the fæces to take on a soft consistency for a period of nine days; the drug did not appear in the urine and only slowly disappeared from the fæces. The other two compounds failed to keep the stools soft for more than a day or two, the last, indeed, proving to be very untrustworthy as a purgative. The prolonged action of the tetrachlor body is due, as we shall see later, to the fact that none of it is lost by escaping in the urine. Phenolphthalein, under the same circumstances, that is, when administered subcutaneously, is excreted to a considerable extent by the kidneys and cannot therefore exert a purgative action for so long a time. The only channel of excretion[25] for the tetrachlor derivative is through the bile, and from this it is reabsorbed to a large extent by the lower bowel.

In table 2 will be found the protocols of further experiments which show that the tetrachlor derivative has a prolonged action as a laxative when injected in oil solution, an action which in the case of dogs lasts from three to nine days, the more usual period being five or six days after a dose of 0.4 gm. These statements are based on a series of thirty experiments on dogs. In no single instance could the drug be detected in the urine, either in the free state or in a state of conjugation. This point is one of great importance when it is recalled that

[25] As we shall see a minute quantity is excreted by the mucosa of the small intestines.

many organic purgatives, more especially those classed as derivatives of anthraquinone, are capable of irritating the kidneys even when administered by mouth[26] in a single medicinal dose, and cause serious lesions in these organs when administered subcutaneously.

TABLE 1

Phenoltetrachlorphthalein

DATE.	CONDITION OF DOG.	PURGATION.	DRUG IN STOOL.	DRUG IN URINE.	
				In conjugated state.	In free state.
10/31....	injected				
11/1.....	normal	+	+	−	−
2.....	"	+	+	−	−
3.....	"	+	+	−	−
4.....	"	+	+	−	−
5.....	"	+	+ slight		
6.....	"	+			
7.....	"	+			
8.....	"	no fæces			
9.....	"	+			
10.....	"	+			

Phenolphthalein

				10–31	+ 2 hrs. after injection.
10/31....	injected				
11/ 1....	normal	+	+	+	+
2....	"	+	+		
3....	"	−	−		
4....	"	no fæces			

Tetrabromphenoltetrachlorphthalein

10/31....	injected				
11/ 1....	normal	− no fæces			
2....	"	+			
3....	"	− no fæces			
4....	"	+			
5....	"	− no fæces			

[26] Marshall: Scottish Med. and Surg. Jour., x, 406, 1902. Dixon: Brit. Med. Jour., ii, 1244, 1902. Pfaff: Trans. Assoc. Amer. Physicians, xxiii, 184, 1908.

TABLE 2
Subcutaneous Injections of Phenoltetrachlorphthalein

DATE.	CONDITION OF DOG.	PURGATION.	DRUG IN STOOL.	DRUGS IN URINE.	
				In conjugated state.	In free state.
11....	injected 0.3 gm.	in 30 cc. oil			
12....	normal	−			
13....	"	+	+		
14....	"	+	+		
15....	"	+	+		
16....	"	+	+		
17....	"	+	+		
1/ 8....	injected 0.4 gm.	in 20 cc. oil			
1/ 9....	normal	+	−	−	−
1/10....	"	+	+		
1/11....	"	+	+		
1/12....	"	+	+		
1/ 8...	injected 0.4 gm.	in 20 cc. oil			
1/ 9....	normal	+	+	−	−
1/10....	"	+	+		
1/11....	"	+	+		
1/12....	"	− no fæces			
1/13....	"	+	+		
1/14....	"	+	+		
1/15....	"	+			
2/12....	injected 0.3 gm.	in 15 cc. oil			
2/13....	normal	+	+	−	−
2/14....	"	+	+		
2/15....	"	+	+		
2/16....	"	− no fæces			
2/17...	"	+	+		
2/18...	"	+	+		
20....	injected 0.2 gm.	in 10 cc. oil			
21....	normal	+	+		
22....	"	+	+		
23....	"	+	+		
24....	"	+	−		
25....	"	+			
26....	"	+			
27....	"	−			
21....	injected 0.2 gm.	in 20 cc. oil			
22....	normal	+	+	−	−
23....	"	+	+	−	−
25....	"	+	+	−	−
26....	"	+	−	−	−

In view of the fact that no harmful results follow upon the hypodermic administration of phenoltetrachlorphthalein to animals, we have ventured to try this purgative in medical practice, administering it in the same manner.

Our first trials (five cases) were made in the obstetrical wards of the Johns Hopkins Hospital, with the coöperation and kind assistance of Professor Williams and Dr. H. J. Storrs, but they were not as satisfactory in their outcome as we could wish. In the light of our later experience we feel sure that this was due to the fact that we did not inject large enough quantities of our oil solution of phenoltetrachlorphthalein. In these first cases we administered only 10 to 15 cc. (0.2 to 0.3 gram), whereas we now find it necessary to use at least 20 cc. (0.4 gram).

After these preliminary trials we began to treat patients suffering from obstinate chronic constipation but who were otherwise healthy and were not confined to bed, by these subcutaneous injections of our chlor derivative. Some of these patients had been returning to the dispensary for relief from constipation for periods varying from months to years, and the majority of them failed to have an evacuation except when taking purgatives. At the present writing[27] twenty patients of this kind have been under treatment, each receiving 20 cc. of neutral olive oil containing 0.4 gram of the drug. In nineteen of the cases the results have been satisfactory. Some of these people have used a glycerine suppository to supply the stimulus for the first or second evacuation in case no movement of the bowels occurred within thirty-six hours after administration of the drug. In the main the results are entirely like those obtained with dogs. The one injection has been followed by daily evacuation (one or two stools per day) for a period varying from five to fifteen days. The average number of movements during the first week was five to seven.

We are well aware that habitual constipation can sometimes be cured or alleviated by attention to diet and exercise, by insistence on regularity[28] in the time set aside for a daily movement, especially

[27] March, 1909. A more detailed report of the use of this phthalein in a larger number of cases will be made later by L. G. Rowntree.

[28] Trousseau long ago remarked that the will alone if persistently applied to the task can cure constipation. Cited from Edinger's Article, *Verstopfung*, in Eulenburg's Real Encyclopäedie der Gesammten Heilkunde, 2te. Aufl., 1890. See also I. P. Lyon: Trans. Amer. Physicians, 1908.

when the patient is profoundly convinced of the efficiency of these measures and applies himself seriously and enthusiastically to the task of carrying out the orders of his physician. But the cases treated by us were chronic cases who had returned time and time again to the dispensary, on whom psychotherapy and advice had never produced any results and on some of whom cascara in the usual doses had no effect.

Our patients were told that the injections would cause them to have a movement each day for a number of days. They were directed to take some fruit each day, to drink freely of water, to exercise, and to go to stool regularly night and morning. It was hoped that the success attending the observance of these rules during the period when daily movements of the bowels were being induced by chlorphthalein, would encourage our patients to continue in the observance of these rules and that they would thus eventually obtain a complete cure without further resort to drugs.

We do not maintain that a single injection of phenoltetrachlorphthalein is effective for more than five or six days in a human being. That it can be effective for that length of time, without assistance from psychotherapy, is proved by our numerous experiments on dogs.

Our experiments in this field point to the phthaleins or allied compounds as a class of substances that will probably furnish us with a serviceable non-toxic hypodermic purgative. The tetrachlor derivative is efficient and non-toxic, but its insolubility in water and its low solubility in oil stand in the way of its application as a hypodermic purgative, although they do not detract from its efficiency where a slowly acting subcutaneous purgative or laxative is indicated. From the evidence at hand it would appear that phenoltetrachlorphthalein, hypodermically administered, is a slowly acting, non-toxic laxative, whose action after a single dose may extend over several days.

IX. ABSORPTION OF THE TETRACHLOR DERIVATIVE AND ITS FATE IN THE BODY.

Absorption of this compound from the area of injection in the subcutaneous tissues proceeds very rapidly. Five dogs have been killed within sixteen to twenty hours after receiving the drug subcutaneously,

and after the expiration of this time only a trace of the drug could be detected in the area of injection. The local swelling, due to the injection of the considerable volume of oil, subsides very quickly after the injection has been made.

When administered by mouth this drug appears to be absorbed to a smaller extent than phenolphthalein, which Vamossy[29] has shown to be recoverable from the fæces to the extent of 85.17 per cent.

It is of interest to inquire in what form the drug circulates when it is administered by subcutaneous or intravenous injection. Its salts with sodium and potassium, like those of phenolphthalein, are decolorized on coming in contact with serum or with the tissues of the body. But this does not imply that these phthaleins exist only in the form of free acids in the tissues. We have seen that both substances are excreted in the bile and in the urine[30] in the "free" and in the conjugated form, both forms being colorless. We may assume that the free phthaleins, their colorless monosodium salts (if such exist) and a conjugated form exist side by side in equilibrium with each other and with the constituents of the tissues.

Vamossy[31] has set up the hypothesis that phenolphthalein is converted to some extent in the intestine into a sodium salt which is only slightly absorbed and which is supposed to act as a purgative by virtue of its high osmotic pressure. Fleig[32] has already pointed out that the assumption that the purgative action of phenolphthalein is dependent on the presence in the intestine of so large an amount of the sodium salt as is required by the osmotic theory, calls for more experimental evidence than has yet been brought to its support.

Fleig[33] has made careful and exhaustive experiments which prove that phenolphthalein undergoes no destructive change in the tissues, that it is recoverable without loss after it has been exposed to the action of minced organs, or when its solutions are made to pass through surviving organs. We have not made similar quantitative experiments with the tetrachlor derivative. When this substance is

[29] Therapie der Gegenwart, 1902.

[30] To obtain the tetrachlor derivative in the urine it must be injected intravenously in rather large amounts.

[31] Loc. cit.

[32] Loc. cit., p. 351.

[33] Bull. d. Sciences Pharmacol., xv, 381–84.

administered by the method of subcutaneous injection, so large an amount is found in the fæces that we feel confident that only a small part, if any, is lost in the body by destructive changes.

X. CHANNELS OF EXCRETION.

It has already been stated that the tetrachlor derivative is never discoverable either in the bile or in the urine when it has been given by mouth, but that phenolphthalein under the same conditions appears in the urine and also in the bile.

When the tetrachlor derivative is given subcutaneously, it escapes from the body only in the bile; the amount that passes into the intestine in other secretions being so minute that it can barely be detected. In proof of this statement the following experiments may be cited:

Experiment I. Dr. S. J. Crowe of the Hunterian Laboratory kindly provided us with a small dog having a permanent biliary fistula. In this animal the bile escaped through an opening in the gall-bladder which had been sewed to the abdominal wall, the common duct having been ligated. The usual quantity for small dogs, 0.4 gram, of the tetrachlor derivative was given subcutaneously, and measures were taken to prevent licking up of bile from the fistulous opening. We cannot be absolutely certain that we were successful in entirely preventing this. Under the conditions of this experiment the bile shows in sixteen hours[34] the presence of an abundance of the drug. It appears in the two forms, the "free" and the conjugated, as already described in speaking of phenolphthalein. After eighteen or twenty-four hours the bile contains very large amounts of the drug. The fæces of the day following the injection did not turn red on the addition of sodium hydroxide, but when they were dried on the water bath and extracted with acetic ether a very minute quantity, a mere trace, of the tetrachlor body could be detected. Nothing in the way of a conjugated derivative could be detected either in the residue from the acetic ether or in the aqueous extract of the dry fæces.

[34] The drug was injected at 5 p.m., the test was made at 9 a.m. on the following day. We cannot state how early the drug appears in the bile in experiments of this kind.

Experiments II, III and IV. In these experiments with dogs the procedure was as follows: Tertiary trichlorbutyl alcohol ("chloretone") was given by mouth in the dose of 0.2 gram pro kg. of body weight. Under anæsthesia by ether the abdomen was opened and a broad ligature was placed on the duodenum below the openings of the biliary and pancreatic ducts. A purgative dose of the tetrachlor derivative, 0.4 to 0.5 gram in oil, was then injected under the skin of the back of the neck. The animals were then placed in a warm room and allowed to remain in the state of unconsciousness produced by the "chloretone," whose action comes on as soon as the influence of the ether subsides. At the end of twenty-four hours animal II was killed, animal III was killed at the end of thirty-six hours, and animal IV survived for twenty-four hours. The intestinal contents of none of these animals could be made to give a test for the tetrachlor derivative by direct addition of an alkali, and on shaking with acetic ether none of the drug could be extracted from the contents of either the small or the large intestine. The application of a five or ten per cent solution of sodium hydroxide to the mucosa of either the large or the small intestine failed to develop a red color. The bile of all of these animals contained a large amount of the drug both in the "free" and the conjugated form.

In a control experiment in which phenolphthalein was used in place of its tetrachlor derivative, a very small amount of the drug could be detected in the intestinal contents by extraction with acetic ether. In this case the urine also contains phenolphthalein in both the "free" and conjugated form, while the urine of dogs II, III, and IV contained none of the tetrachlor derivative.

When a solution of the tetrachlor derivative (50 to 100 cc. of a 5 per cent solution of the disodium salt) is injected into the saphenous vein of a dog and his vascular system is thus overloaded with this substance, the kidneys soon yield and allow it to pass into the urine. Under these conditions, too, the drug is excreted to a small extent in the pancreatic juice, in the succus entericus, saliva, and lachrymal fluid.[35] It may also be mentioned in regard to the tetrachlor deriva-

[35] In these experiments also the liver is the main excreting organ. In ten minutes bile collected from a canula fastened into the common duct will show the presence of the drug in the conjugated form; after the lapse of twenty minutes the drug begins to appear in the "free" state.

tive that it is not excreted in the milk when administered subcutaneously. This point was established in a few trials that were made with it in the obstetrical wards of Prof. J. Whitridge Williams.

XI. REABSORPTION BY THE LARGE INTESTINE

Experiments I, II, III, IV and V. In these experiments varying quantities of the tetrachlor derivative, either in the form of an oil solution (20 to 30 cc. of 2 per cent solution) or in the form of the aqueous solution of the disodium salt, were injected into dogs subcutaneously. After the expiration of from eighteen to thirty hours the animals were killed, the large and the small intestines were removed separately, opened, and washed perfectly clean under running water, the fingers being used to rub away adherent bile and other material. On the application of a five or ten per cent solution of sodium hydroxide to the mucous surface of the large intestine, the mucosa of this organ is observed to take on an intense, brilliant and deep-red stain throughout its whole extent. The color cannot be removed by washing the mucous membrane under the tap, and it is evidently the expression of a thorough and uniform saturation of the mucosa by the tetrachlor derivative. No color reaction can be obtained by applying alkali to the mucous surface of the small intestine. If by any chance a streak of red color should be observed anywhere in the small intestine, it will be found that it is due to an adherent patch of bile-stained mucus which was not washed away and which is easily removed by rubbing with the finger, showing the unstained and intact mucosa underneath. The brilliant deep-red color begins to appear abruptly at the line where the small intestine merges into the large gut.

In order to submit this question of the saturation of the mucosa of the large intestine to further tests and to meet the possible objection that this phenomenon results from the uniform deposition of the phthalein on the surface of the mucosa, we proceeded as follows. Three dogs that had received the drug subcutaneously in doses ranging from 0.4 to 1 gram, were killed at the expiration of from 24 to 36 hours. The intestines were washed repeatedly with sufficient quantities of an 0.85 per cent solution of sodium chloride, until all traces of bile and fæcal matter were removed. During this process

the mucosa was rubbed gently with the fingers and when absorbent cotton was used care was taken not to apply it too vigorously. Frozen sections (of considerable thickness) from both the large and small intestines were then made and examined under the binocular microscope. Only sections taken from the large gut gave evidence of the presence of the drug on the addition of sodium hydroxide. In these sections the drug was found not only in the cells of the mucosa but also in the submucosa, as indicated by the appearance here of a red band at the moment of contact with the alkali.

The mucosa of the large intestine may, of course, be thoroughly denuded of its superficial cells by too vigorous rubbing with absorbent cotton, especially, if during this operation the intestine is so stretched as to obliterate its folds and in this event little or none of the phthalein will be found in the mucosa of the section. But even under these conditions we have found that the drug is present in the submucous tissue. In one experiment ox-bile, which is a good solvent for our phthalein, was employed in washing a small piece of the large gut, but here also the mucosa of the cleaned piece gave a brilliant reaction with alkali.

Control experiments in which solutions of phenolphthalein in oil were administeed subcutaneously show that this drug behaves in the same manner and gives an equally intense red color when the mucosa of the large intestine is moistened with a solution of sodium hydroxide.

This phenomenon cannot, however, be demonstrated with either phenolphthalein or its tetrachlor derivative in experiments in which these drugs are given by the mouth. Here, as in the case of the small intestine, a streak of red color or minute dots and patches of red color can now and then be obtained on the addition of alkali to the washed mucosa, but careful inspection will always show that these are due to adherent particles of bile-stained fæces.

To prove that this interesting phenomenon is due to reabsorption of the drug from the bile and is not the result of excretion by the mucosa of the large intestine, one has but to make the simple experiment of ligating the small intestine just below the orifices of the pancreatic and biliary ducts and again give the drug (either phenolphthalein or its tetrachlor derivative) subcutaneously as before. As in previous

experiments of this kind, the animal is kept deeply under the influence of chloretone during the eighteen or twenty-four hours' sleep that intervenes between the time of the injection and the time when the intestines should be excised and examined. In this case not a trace of color can be brought out on the mucous surface of the large intestine by the application of an alkali. The drug is in this case retained in the gall-bladder and liver. It has already been stated that when a solution of the drug is injected under the skin of a dog having a permanent biliary fistula, only a minute quantity of it can be extracted from the dried fæces with acetic ether. But here, as has been pointed out, we cannot be certain that licking of the opening in the abdominal wall was entirely prevented.

In addition to the above, two further experiments were made to learn whether the tetrachlor derivative is readily absorbed by the large intestine. Solutions in oil and aqueous solutions of the sodium salt were given in the form of an enema to a dog with a permanent biliary fistula. It was found that the drug is readily absorbed when administered in this way and in these solutions, as is proved by the fact that the bile contains the drug in abundance some hours after the injection.

We have then in phenoltetrachlorphthalein when administered subcutaneously a striking example of excretion by a single organ—the liver, and of reabsorption from its solution in bile by a single organ—the large intestine. When the drug is injected subcutaneously, the sequence of events is as follows: Fairly rapid absorption from the place of injection, transportation to the liver, where in all probability the synthesis of the conjugated fraction is effected, and where both this and the so-called "free"[36] fraction are excreted with the bile. The work of McFadyen, Nencki and Sieber[37] has shown that in the case of the human being the bile salts are not entirely absorbed in the small intestine but pass into the large intestine in considerable amounts, and the same condition probably obtains in the digestive tract of the dog. Test-tube experiments show that solutions of the bile salts

[36] The term "free" is not intended to exclude the existence of colorless monobasic salts and is merely used to indicate that a given secretion contains the drug in a form which reacts with alkalies without previous hydrolysis.

[37] Archiv f. exp. Pathol. u. Pharmakol., xxviii, p. 311, 1891.

obtained from ox-bile act to some extent as solvents for our phthaleins. We must conclude that our tetrachlor derivative is carried into the large intestine in a state of true solution. *That portion of our drug which is absorbed by the large intestine is again excreted by the liver, again absorbed in large part by the mucosa of the large intestine, with a repetition of this cyclic process, as long as the drug remains in the system.* Repeated observations have shown us that it may be detected in the fæces on the addition of sodium hydroxide for four and sometimes five days after subcutaneous injection. In the case of a dog with a biliary fistula, however, the same amount of the drug per kg. of body weight given in the same way disappears from the bil at the end of the second day or beginning of the third.

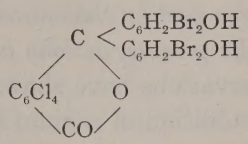
We have not been able to convince ourselves that the drug has a laxative or purgative effect when given hypodermically to an animal with a biliary fistula. When, however, the bile is allowed to carry the drug to the lower bowel, as in our ordinary experiments, a laxative effect is experienced throughout the entire period of the excretion-reabsorption cycle.

Phenolphthalein differs from the tetrachlor derivative in that it does not exert so prolonged an action when given subcutaneously. This is primarily due to the excretion of this drug by the kidneys, perhaps also to a more incomplete absorption from the lower bowel. As far as its affinity for the liver and the large intestine is concerned, it behaves in general like its tetrachlor derivative, and when it is desired to give a demonstration of this striking example of selective absorption, it may well be used in case the tetrachlor derivative is not obtainable.

We are unable to state why the mucosa of the small intestine is powerless to attract these phthaleins to itself, or why the mucosa of the large intestine and the cells of the liver have so marked an affinity for them, but we hope to offer an hypothesis in explanation of these phenomena in a future paper. The fact that so large an amount of these bodies can be stored in the absorbing structures of the large gut makes it appear probable that these structures contain one or more substances that have an especial affinity for these phthaleins. It is evident, too, that the colorless combination of phthalein and hypothetical substance must be capable of existing in a state of equilibrium

with those compounds which regulate the carbonic acid tension of the tissues. The problem presents an interesting and special case of selective affinity and we intend to give it more careful study.

Tetrabromphenoltetrachlorphthalein,



This substance occurs as a slightly brownish, almost white crystalline powder, very little soluble in water, but more soluble in olive oil than either phenolphthalein or its tetrachlor derivative. Dilute solutions in alkalies have a blue color.

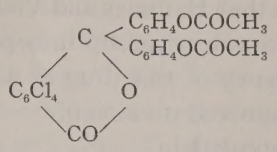
Numerous experiments were made on dogs to test the efficiency of this drug as a purgative. Administered by mouth in doses of one gram it was found to exert a mild laxative action comparable to that induced by phenolphthalein or its tetrachlor derivative. Used hypodermically, however, in oil solution in doses of 0.5 to 1 gram it is less effective as a purgative than tetrachlorphenolphthalein.

Even when administered subcutaneously in considerable doses this compound is not excreted by the kidneys. Under these circumstances it is found in the fæces and here its presence is made evident by the addition of an alkali which causes an extract of the fæces gradually to take on a green color. Inasmuch as this phthalein itself takes on a blue color on the addition of the hydroxides of the alkalies, it is of interest to inquire why the excreted product should show a change of color from blue to green under the same treatment. It was found that when the blue solution of an alkaline salt of the phthalein was added to a dilute solution of bilirubin in sodium hydroxide a fine green color (entirely like that obtained by the addition of alkali to fæces as stated above) made its appearance. The same change of color is observed when the blue solution is added to a dilute solution of bile.

Furthermore, when an aqueous extract of fæces from a dog that has not received this drug is rubbed up with this phthalein and filtered, it will be found to give a green color on the addition of an alkali. These experiments prove that the observed color reaction is due to

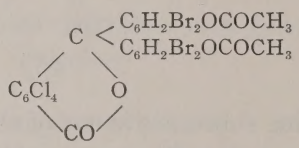
the presence of biliary constituents and does not indicate that the drug has been altered in its passage through the body.

Phenoltetrachlorphthalein diacetate



and

Tetrabromphenoltetrachlorphthalein diacetate,[38]



These substances are more soluble in oil than the corresponding non-substituted compounds, and it is therefore possible to inject subcutaneously doses of one gram or more without using more than 20 cc. of oil. In none of our experiments, all of which were made on dogs, could we obtain satisfactory evidence that these compounds act as purgatives. Administration by mouth to dogs, in doses of 1 to 2 grams, also fails to induce a laxative effect.

We have made no experiments to determine the fate of these derivatives in the animal organism, and can only say that they are not excreted in the urine and that they appear in the fæces when they are given subcutaneously.

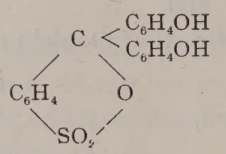
It may be taken for granted that these diacetates are excreted by the liver when they have been administered subcutaneously, but to what extent reabsorption takes place in the intestinal tract has not been determined. An aqueous extract of the fæces always gives a more or less marked color reaction on the addition of sodium hydroxide, which fact proves that a very small percentage only of these diacetates undergoes hydrolytic cleavage in the intestinal tract.

It is evident that the purgative action of these phthaleins for dogs is greatly weakened by substitution in the hydroxyl groups, and

[38] For a description of these substances see Orndorff and Black: Am. Chem. Journ., xli, 349, 1909.

their inefficiency is accounted for by the fact that hydrolytic cleavage, with regeneration of the original phthaleins, occurs to so limited a degree in the digestive tract of these animals. It may be mentioned in this connection that Hammer and Vieth[39] find that the introduction of an acetyl and a valeryl group into phenolphthalein has in nowise lessened the efficiency of this drug as a purgative for human beings but has rather improved its action.

Phenolsulphonephthalein,



This interesting substance is one of a class of compounds analogous to the phthaleins and was first prepared by Ira Remsen.[40] As described by his pupil, Sohon,[41] this substance is a bright red crystalline powder somewhat soluble in water, more so in alcohol, insoluble in ether; its dilute alkaline solution is of a purer red than that of phenolphthalein, while a more strongly alkaline solution is purple. It is readily soluble in solutions of sodium carbonate and has a stronger avidity as an acid than any of the phthaleins hitherto examined by us.

Solutions of[42] the sodium salt properly made up may be injected under the skin without the slightest evidence of an irritant action. The drug may also be administered by mouth without untoward effects of any kind. Taken into the mouth as a powder it is at first slightly sweetish to the taste, then bitter and rather disagreeable.

[39] Aperitol (valeryl acetyl phenolphthalein) Mediz. Klinik, 1908, no. 37. We have seen only the brief review concerning this purgative which appears in the Zentralbl. f. die ges. Physiol. u. Pathol. des Stoffwechsels.

[40] Amer. Chem. Jour., vi, 180.

[41] Amer. Chem. Jour., xx, 257.

[42] These solutions were prepared as follows: One gram of the sulphonephthalein and 1.4 cc. of 2 N sodium hydroxide were added to 18.6 cc. of either $\frac{N}{2.3}$ sodium chloride solution or distilled water. This gives a solution of the mono-sodium, or acid salt, which is red in color and which if injected in this form produces slight local irritation. It is therefore necessary to add from five to ten drops more of 2 N sodium hydroxide solution, a quantity of alkali which is sufficient to change the color to a beautiful Bordeaux red, and entirely to counteract the irritating effect of the acid salt but insufficient to form the purplish red neutral salt of the phthalein.

Applied in this way it stains the mucous membrane of the mouth and tongue a brilliant red which later becomes yellow and then disappears. Given by mouth to healthy human beings in doses of 0.1 to 0.15 gram the drug is readily absorbed and appears in the urine in the course of an hour or an hour and a half. The urine, if acid, assumes a yellow or reddish-yellow color, which immediately gives place to the purple of sulphonephthalein on the addition of an alkali. After injection underneath the skin in doses of 1.6 cc. of a 5 per cent solution it may be detected in the urine of healthy individuals within ten minutes.

Excretion in the Bile and Urine and Absorption from the Bile in the Intestine

When one gram of phenolsulphonephthalein is administered subcutaneously to a dog of 15 kg. a very small quantity only is discoverable in the fæces, and the urine is apparently the only channel of excretion. We have here a result which is the opposite of that observed in the case of phenolphthalein and its halogen substitution products, which it will be recalled are always excreted in the fæces without reference to their point of entrance into the body. In order to learn whether this sulphonephthalein is excreted in the bile a dose of 1 gram was administered subcutaneously to a dog with a permanent biliary fistula. In less than two hours the bile flowing from the fistula assumed a deep-red color. A single drop of bile sufficed to give a fine purple color to 25 cc. of water containing a drop of sodium hydroxide solution, thus proving that this phthalein is excreted freely in the bile.

Inasmuch as but little of the drug can be detected in the fæces when it has been given subcutaneously, it is evident that the portion of the drug which is excreted in the bile must be absorbed in its passage through the intestines. *A number of experiments were made in which animals were killed at varying periods after subcutaneous administration of the drug, but in no case was it possible to obtain evidence of a selective absorption by one part only of the intestinal tract as in the case of phenolphthalein and its tetrachlor derivative.* All of the divisions of the intestine lying below the orifice of the common bile duct apparently absorb this drug as opportunity offers.

As the drug is excreted by the kidneys only it becomes necessary to inquire whether these are injured in any way by the drug. We have examined carefully the urine of six dogs that had received this phthalein subcutaneously in doses of one gram and have not been able to detect albumin, sugar, casts or other abnormalities.

Injected intravenously into rabbits in doses of from .6 to 1 gram in 10 per cent solution phenolsulphonephthalein exerts a mild diuretic action. This point was established in a number of experiments which were made on anæsthetized rabbits into whose bladder the ordinary bladder cannula had been introduced. Control injections of 0.85 per cent sodium chloride solution were made from time to time.

Phenolsulphone phthalein exerts only a slight (or doubtful) action as a purgative when given by mouth to dogs in doses of one gram, and is entirely devoid of action in this way when administered subcutaneously in this dose.

GENERAL SUMMARY

I. Phenolphthalein and its halogen substitution products, phenoltetrachlorphthalein and tetrabromphenoltetrachlorphthalein, do not differ markedly in pharmacological behavior. Phenolphthalein and its tetrachlor derivative, phenoltetrachlorphthalein, have been compared in respect to their pharmacological properties, and it has been found that they have the following points in common:

They are non-irritant when applied to mucous membranes or to open wounds or when injected subcutaneously in oil solution. Their salts with sodium and potassium are, however, highly irritating when administered subcutaneously in aqueous solution. The toxicity of these phthaleins is very low; large quantities may be injected repeatedly into a vein of the dog without causing any discoverable pathological lesions. They have no bactericidal action. Large doses when given intravenously cause a small and rather prolonged rise of arterial pressure.

II. Both phenolphthalein and its tetrachlor derivative exert a laxative or purgative action when given by mouth, when injected under the skin, or into a vein. *When a solution of the tetrachlor derivative* (0.4 gram) *in oil is injected under the skin of dogs or of human*

beings a laxative action is induced which continues from four to six days. Phenolphthalein administered in the same dose and in the same way does not act for so long a time, for the reason that it is more quickly excreted. This prolonged action together with the low toxicity of the drug leads us to believe that a serviceable hypodermic purgative will be found amoung the phthaleins or their derivatives. The tetrachlor compound is efficient as a hypodermic laxative, but its insolubility in water and its low solubility in oil stand in the way of its general adoption, although they do not detract from its efficiency when a subcutaneous laxative is required.

III. *Excretion.* When the tetrachlor derivative is given *subcutaneously* it escapes from the body *in the bile only,* the amount that passes into the intestine in other secretions being so minute that it can barely be detected. When phenolphthalein is administered in the same way a part of it always escapes in the urine. When the tetrachlor derivative is given *by mouth* none of it appears in the bile or in the urine, but when phenolphthalein is given in this way it may appear in both of these secretions to a small extent.

These phthaleins are excreted in the bile in two forms, in the free and in the conjugated form, and it is this latter form that makes its appearance first in the order of time.

IV. *Absorption by the large intestine.* We have proved in several ways that after subcutaneous administration of phenolphthalein and its tetrachlor derivative the mucosa of the large intestine absorbs these drugs from their solution in bile, and *becomes so thoroughly saturated with them that it assumes a deep-red color upon being moistened with a solution of sodium hydroxide.* The mucosa of the small intestine cannot be made to give a color reaction for phthaleins, although a stream of bile containing these drugs may have passed over it for many hours. It has been shown also that these phthaleins are not excreted by the intestines except in minimal quantity if at all.

V. Phenolsulphonephthalein agrees with the phthaleins just named in having a low toxicity and in being excreted in the bile, but differs from them in being more completely reabsorbed from the intestine.

In this case all of the divisions of the intestine absorb the drug as opportunity offers, the upper portions, being nearer to the orifice of the common bile-duct, naturally absorb more than the lower portions.

Irrespective of the manner of its administration this phthalein is rapidly eliminated from the body by the kidneys. Administered intravenously to rabbits in doses of 0.5 gram it has a slight action as a diuretic. Administered by mouth to dogs in doses of one gram it exerts only a slight action as a laxative; administered intravenously in these doses it is entirely devoid of action. The presence of a sulpho group (SO_2) has increased the acidity and the power of salt formation of this phthalein and it may be assumed that these properties render the drug excretable by the kidneys. The entrance of a sulphonic acid group (HSO_3) into phenoltetrachlorphthalein would no doubt effect similar changes in its physical and pharmacological properties.

VI. The introduction of the acetyl radical into the molecule of phenoltetrachlorphthalein and into that of tetrabromphenoltetrachlorphthalein has lowered the efficiency of these drugs as laxatives for dogs. This change is probably due to the insolubility of these compounds and also to the fact that they are not saponified to an appreciable degree in the alimentary tract.